

FLUORESCENT MAILLARD REACTION ARTIFACTS IN PLANT EXTRACTS

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ABSTRACT.—A discussion based on literature references is presented which describes the possible formation of fluorescent artifacts by the Maillard reaction during the usual procedures for extraction and isolation of fluorescent secondary plant substances.

Reactions of the amino group of amino acids with sugars has been of particular interest to the food industry and has been the subject of extensive investigations. This reaction, called among other things the Maillard reaction and non-enzymic browning, has been reviewed (Ellis, 1959; Reynolds, 1963, 1965). However, it has not received much attention by plant physiologists, pathologists, or taxonomists. The purpose of this paper is to indicate the possible importance of the reaction to investigators conducting research involving chromatographic separations of fluorescent substances in plant extracts.

As an example of the possible importance of this reaction, in investigations of chemical factors involved in plant disease resistance, plant tissue is usually extracted with boiling ethanol or sometimes with hot water followed by extraction of the aqueous solution with an organic solvent such as ether. Potassium bisulfide and metabisulfite are frequently added to prevent darkening of the extracts. The extracts are then chromatographed and many of the secondary plant substances are located by their fluorescence under ultraviolet radiation.

In addition to some phenolic compounds and many other UV-fluorescing compounds, fluorescence has also frequently been observed in sugar-amino acid reaction mixtures, but the compound(s) responsible for this property has not been identified. For example, refluxing of solutions (0.1-1M) of D-

glucose and glycine resulted in at least 10 fluorescent compounds when examined by paper chromatography (Patton and Chism, 1951). Although most workers would consider this a large number of possible artifacts, glycine is not the best producer of fluorescent compounds; tryptophan, histidine, phenylalanine, threonine, lysine, and glutamic acid were demonstrated to be better (Friedman and Kline, 1950; Shore and Pardee, 1956).

Bisulfite is often used to prevent the loss of phenols during extractions. However, the presence of bisulfite not only retards browning but promotes the accumulation of fluorescent compounds in heated mixtures of amino acids and sugars (Friedman and Kline, 1950; Overby and Frost, 1952; Shore and Pardee, 1956). The formation of these compounds has a pH optimum between 6 and 8 (Shore and Pardee, 1956) and is temperature (Pearce, 1950) and moisture (Pearce, 1949) dependent. Experiments conducted in this laboratory confirm these observations. When a mixture of amino acids and fructose was heated in the presence of bisulfite, a mixture of fluorescent compounds was produced. These synthetic fluorescent compounds were absorbed on cyanide-deactivated carbon and eluted with benzene-methanol-water (3:7:1 v/v) as used in the isolation of a glycoside fraction from corn stalks (Wahlroos and Virtanen, 1959; BeMiller and Pappelis, 1965). They are chromatographically similar to some of the compounds extracted by ether from a pH 2 solution obtained from a water extract of corn stalk tissue on which natural glycosidases have acted (BeMiller and Pappelis, 1965a).

The synthetic compounds obtained from amino acids and fructose do not show reactions with spray reagents similar to those of the phenolic acids; for example, they do not exhibit in-

creased fluorescence when sprayed with 5% sodium carbonate.

It is not known whether or not any of the compounds in corn stalk extracts which are similar chromatographically and by fluorescent reactions to the synthetic compounds are indeed reaction products of amino acids and sugars. Even if they are, it is not known whether they are naturally occurring compounds like the amino acid-chlorogenic acid addition compound (Clark et al., 1959) or artifacts produced during hot water or hot ethanol extraction and subsequent concentration.

From the literature discussed here, however, it appears that the extraction conditions commonly used with plant materials are ideal for artifact formation and it is quite possible that such compounds will be present in these extracts. Since they could become part of various purified fractions, they could interfere with the location of naturally occurring fluorescent compounds. Hence, these compounds formed by the Maillard reaction need further study, especially by plant physiologists, pathologists, and taxonomists conducting chromatographic separations or similar studies of fluorescent substances in plant extracts.

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